MANUFACTURING OF DRUG-PLGA MICRO- AND NANOCOMPOSITES BY SUPERCRITICAL CO_2 EXTRACTION OF EMULSIONS

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Supercritical Fluid Extraction of Emulsions (SFEE) is used for producing nanoparticles of the biopolymer poly-lactic-co-glycolic acid (PLGA). Specifically, the formation of drug-PLGA composite particles is investigated in this study. The two compounds lysozyme (hydrophilic) and ketoprofen (hydrophobic) have been incorporated in PLGA using specific encapsulation strategies accounting for their chemical properties. Co-formulation products were analyzed for morphology, drug content and stability. The current study highlights the potential of SFEE as an attractive and scalable process for the manufacturing of drug-PLGA composite particles for pharmaceutical applications.

INTRODUCTION

The biocompatible and biodegradable polymer poly-lactic-co-glycolic acid (PLGA) is a promising constituent for drug-polymer co-formulations serving specific pharmaceutical purposes, such as controlled release and targeted drug delivery [1]. For these applications, the size of co-formulation particles is an important parameter, determining for instance the kinetics of drug release from the particles, or their suitability for drug targeting [2, 3]. On the other hand, an efficient encapsulation of the active pharmaceutical ingredient is required, leading to the formation of highly pure drug-polymer composites exhibiting a sufficient long-term stability while maximizing the yield. Hence, there is a general interest in scalable processes that enable the efficient production of such composite particles in the size range of a few micrometers or below, and at conditions that comply with the delicate nature of the materials involved.

Supercritical fluid extraction of emulsions (SFEE) is a novel process for the production of PLGA micro- and nanocomposites [4-6]. In this process, an organic solution of PLGA is first dispersed in an oil-in-water emulsion and stabilized by a suitable surfactant. Then, the emulsion is continuously mixed with supercritical CO_2 in order to extract the organic solvent from the emulsion droplets. Thereby, stable suspensions of solvent-free PLGA droplets in water are obtained, and upon depressurization, the solidified polymer particles may be recovered. Different possible approaches to encapsulate an active pharmaceutical ingredient into PLGA are shown in Figure 1. The left column indicates the raw materials used for encapsulation. PLGA is always used in solution, while the drug may be added either as solid or as a solution. Drug incorporation into the PLGA solution is achieved as shown in the middle column of Figure 1. In the simplest case, drug and polymer form a fully homogeneous solution in a common solvent (Figure 1, top). If otherwise the drug is insoluble in the PLGA solution, it may be dispersed therein as finely pulverized solid, thus forming a suspension (Figure 1, center). If the drug can be neither co-dissolved with PLGA nor obtained in a sufficiently micronized form, it may be dissolved in a different solvent before mixing with the

PLGA solution. There are now two possibilities: First, the two solutions may be immiscible, thus forming an emulsion when mixed (Figure 1, bottom). Second, if the two solutions are miscible, the drug is likely to precipitate and form a suspension in situ (Figure 1, center). The right hand side of Figure 1 shows how the aforementioned drug-polymer dispersions are transformed into emulsions, as required for particle formation by SFEE. Depending on whether the drug has been incorporated into a solution, a suspension or a w/o-emulsion, the final mixing step yields an o/w emulsion, an s/o/w suspension emulsion or a w/o/w double emulsion.

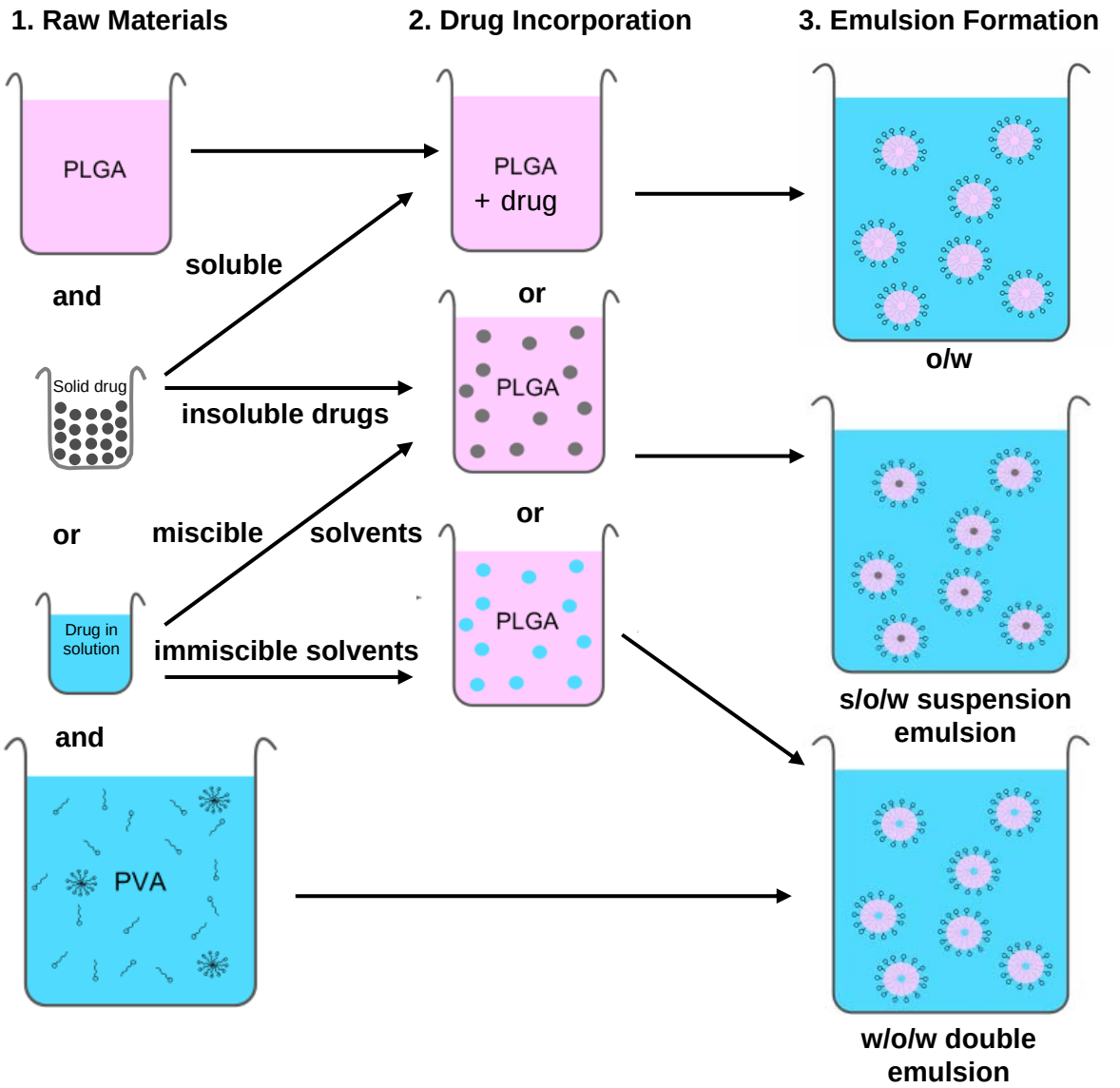

Figure 1: Different approaches for drug encapsulation into PLGA

In the scope of this study, we want to present an experimental study on drug-PLGA co-formulation, comprising the encapsulation of two different compounds, namely lysozyme (LYS), i.e. a typical water-soluble biomolecule, and the non-steroidal anti-inflammatory drug ketoprofen (KET), i.e. a typical hydrophobic drug of small molecular weight.

MATERIALS AND METHODS

Materials: Carbon dioxide (CO_2; 99.9%, PanGas, Schlieren, Switzerland), poly(lactic-co-glycolic) acid 5050 DLG 5A (PLGA; Lakeshore Biomaterials, Birmingham AL, United States), poly(vinyl alcohol) 4-88 (PVA), hen egg white lysozyme, dichloromethane (DCM), ethyl acetate and dimethyl sulfoxide (DMSO, all from Sigma-Aldrich, Buchs, Switzerland) were used as received from the suppliers.

Emulsion preparation: PLGA was dissolved in ethyl acetate saturated with water, and a 1%wt. solution of PVA was prepared in water saturated with ethyl acetate. The organic solution (20 g) and aqueous PVA solution (80 g) were used to form an o/w emulsion upon mixing with a Polytron mixing device (Kinematica AG, Luzern, Switzerland). The stirring rate was adjusted as specified for each experiment, and stirring was applied for 2 min. In order to incorporate water-soluble lysozyme in PLGA particles, the following modified methods were used for the preparation of the organic PLGA solution.
w/o/w Double Emulsion method: Lysozyme was dissolved in water, and PLGA was dissolved in ethyl acetate. Both solutions formed a w/o emulsion upon mixing at high stirring rates. This primary emulsion was then used as described previously for the PLGA solution to prepare a w/o/w double emulsion.
s/o/w Suspension Emulsion method: For the preparation of the suspension-emulsion, solid lysozyme nano-particles were prepared by a supercritical CO_2 antisolvent precipitation step as described elsewhere [7]. These lysozyme particles were dispersed in a solution of PLGA ethyl acetate to form a suspension, which was then used as described previously for the PLGA solution to prepare a s/o/w suspension emulsion.
s/o/w In situ Suspension Emulsion method: A solution of lysozyme in DMSO was mixed with a solution of PLGA in ethyl acetate. Since ethyl acetate is miscible with DMSO but it is an anti-solvent for lysozyme, the latter precipitated and forms solid particles within the PLGA solution. The suspension thus formed was then used as described previously for the PLGA solution to prepare a s/o/w suspension emulsion.

SFEE - Experimental Setup and Procedure: A scheme of the experimental setup used for SFEE experiments as well as a detailed description of the experimental procedure is available elsewhere [8]. Briefly, the emulsion feed stream (2 ml/min) was mixed with a CO_2 feed stream (80g/min) in a mixing nozzle located at the entrance of a 1-litre vessel filled with $scCO_2$ at operating conditions (80bar, 45°C), thus extracting the solvent from the emulsion. The latter was collected at the bottom of the reactor and recovered at the end of the process. The CO_2 was vented together with the solvent through an exit at the top of the reactor.

Scanning Electron Photomicrographs: For sample preparation, particles were recovered from the suspension and separated from the surfactant PVA by centrifugation. The supernatant was discarded, and sedimented particles were re-suspended in pure water and subsequently freeze-dried. Samples were sputter-coated, and SEM photomicrographs were obtained in order to determine the morphology of the precipitated particles.

Lysozyme Content [8]: Dried particles were dissolved in DCM, and water was added to extract lysozyme from the solution by vigorous agitation. Subsequently, the lysozyme content of the aqueous supernatant was determined by high-pressure liquid chromatography.

Ketoprofen Content [9]: Prior to recovery, centrifugation was used to separate the polymeric co-formulation particles from the larger KET crystals that were present in some cases. Particles were recovered, freeze-dried and dissolved in DMSO. The KET content was obtained by UV spectroscopy at 300 nm, i.e. at conditions where PLGA does not absorb light.

RESULTS

The encapsulation of lysozyme into PLGA was investigated in three experiments [8]. Each approach used a different strategy for embedding lysozyme in the polymer solution during emulsion preparation. Namely, these were the w/o/w double emulsion method (run L1), the s/o/w suspension emulsion method (run L2) and the s/o/w in situ suspension emulsion method (run L3). With the double emulsion method, reasonable encapsulation efficiencies are only achieved if the droplets of lysozyme solution in the primary w/o emulsion are much smaller than the particle-forming organic droplets in the final double emulsion. Hence, the final w/o/w emulsification step in run L1 was carried out at a lower stirring speed (3,700 rpm) as compared to the formation of the primary w/o emulsion (30'000 rpm). On the other hand, the final emulsification step was carried out at a high stirring speed (30,000 rpm) in runs L2 and L3. After SFEE processing of emulsions, recovered product particles were freeze dried and analyzed for lysozyme. Two key results may be derived: First, the loading, i.e. the measured fraction of lysozyme in the sample; second, the encapsulation efficiency, which is the ratio of the measured loading and the maximal attainable loading corresponding to the amounts used in each experiment, namely 100 mg lysozyme and 2 g PLGA. High drug loadings of 1.76 % and 2.31 %, corresponding to encapsulation efficiencies of 37.0% and 48.5%, were achieved in experiments L2 and L3, respectively, when solid lysozyme precipitates were used for encapsulation. On the other hand, a relatively low loading of 0.52%, corresponding to an encapsulation efficiency of 10.9%, was achieved in run L1 using the double emulsion method. Images of particles produced in runs L1 and L3 are shown in Figure 2. It may be seen that due to the low stirring rate, the double emulsion method yields larger particles of rather irregular size (Figure 2a) as compared to the product of run L3 which was obtained using a much higher stirring rate during emulsion preparation (Figure 2b).

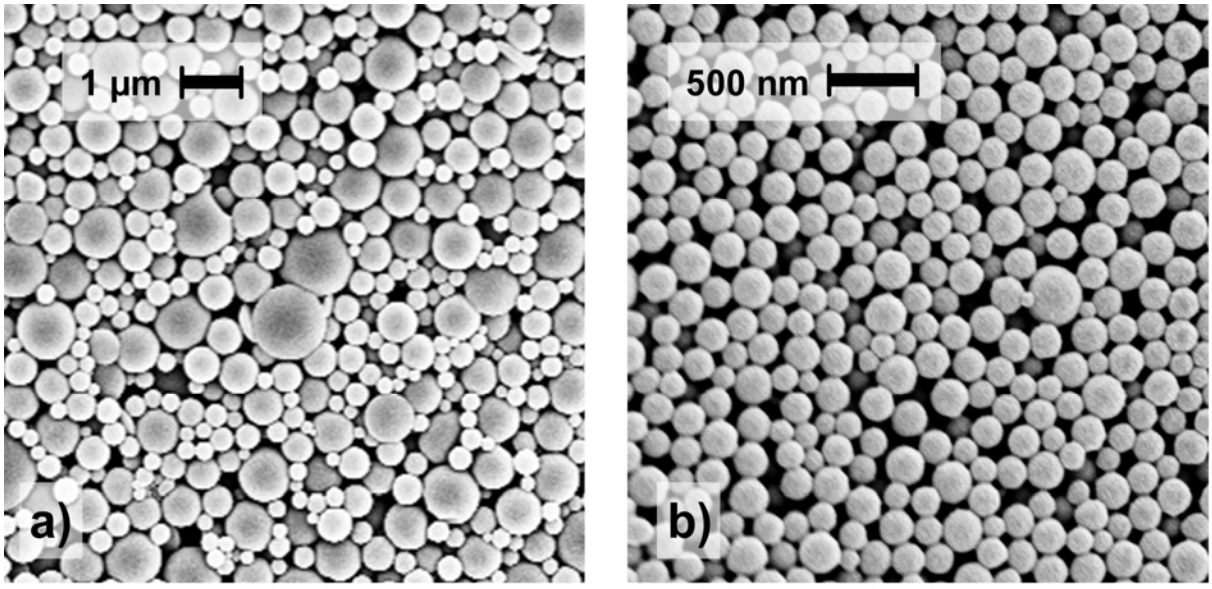

Figure 2 : Photomicrographs of PLGA-lysozyme composite particles achieved in runs L1 (a) and L3 (b).

The co-formulation of KET and PLGA by SFEE was investigated in a series of experiments where the nominal KET to PLGA ratio was varied [9]. After SFEE processing of emulsions, the product particles suspended in water were stored, and samples were taken during one week. Finally, centrifugation was used to separate the polymeric co-formulation particles from the larger KET crystals. Recovered product particles were freeze dried and analyzed for KET. Figure 2 reports the results of this stability analysis, plotting the KET content of composite particles versus the sampling time. At high ratio of KET to PLGA, a decreasing KET content of composites was observed while large KET crystals formed in the suspension (□). In other cases, KET seed crystals were deliberately added to the suspension (○,■).

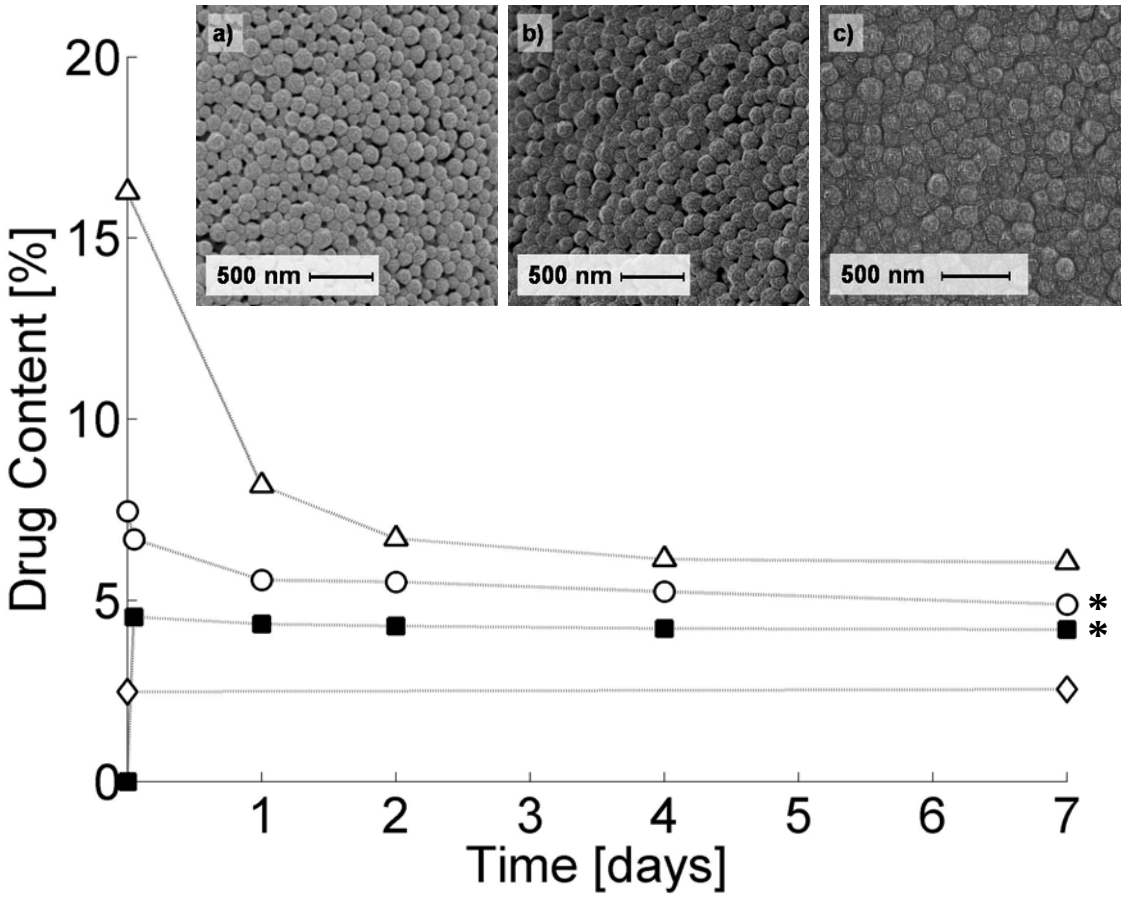

Figure 3: KET-PLGA co-formulations – photomicrographs of composite particles with KET contents of a) 4.5%, b) 6.1% and c) 14.3% (top). Results of stability observation during one week of storage (bottom). Experiments where KET seed crystals were added are marked with an asterisk.

Remarkably, the addition of seed crystals may cause a decrease (○) as well as an increase in drug content (■, impregnation of initially neat PLGA particles). Thus, co-formulations and also particles of pure PLGA finally attain comparable KET levels when equilibrated with KET crystals. This has been rationalized by assuming that co-formulations represent a single phase and that there is a certain level where KET dissolved in PLGA is in equilibrium with crystalline KET, i.e. a solubility of KET in PLGA. Finally, the stable region with KET levels below the solubility limit is indicated by the experiment exhibiting the lowest KET content (◊), which is constant during the observed period of time. Generally, the initially measured KET content was in all cases by about one third lower than the nominal ratio of KET and PLGA in the emulsion. This indicates a certain loss of KET during the process, i.e. by solubility in the supercritical mixture of CO_2 and solvent.

CONCLUSION

Supercritical CO_2 Extraction of Emulsions has a great potential as process for the production of solvent-free PLGA particles since there are many advantages arising from the application of $scCO_2$ as solvent-extracting agent. For instance, high product purity and a low content of residual solvents may be achieved at the same time, at moderate operating temperatures and with reasonable CO_2 consumption [24]. Thus, compared to conventional extraction or evaporation processes, SFEE constitutes an easily scalable and adaptable unit operation.

Concerning the encapsulation of lysozyme, we have demonstrated that the choice of the encapsulation method has a considerable influence on the attainable encapsulation efficiency, and also on the size of product particles. The latter was only 11% when the double emulsion method was applied, and increased to almost 50% for the in-situ suspension emulsion method that also yielded much smaller composite particles.

For the incorporation of KET into PLGA particles, we have shown that a certain level of KET dissolved in PLGA is in equilibrium with crystalline KET, i.e. there is a solubility of KET in PLGA. For pharmaceutical manufacturing, the determination of solubility is of major importance since it delimits the range in which the production of thermodynamically stable products is possible. Further, it has been shown that KET-PLGA co-formulations may be achieved by common SFEE processing as well as by impregnation of KET into pure PLGA particles. The latter constitutes a safe and surprisingly also fast process for the formation of stable co-formulations, and it may also help increasing the reduced KET yield observed in SFEE processing of KET-PLGA co-formulations.

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